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### REMARKS

The foregoing amendments and these remarks are in response to the Office Action dated February 25, 2003. This amendment is filed with a request for a three month extension of time and authorization to charge Deposit Account No. 50-0961 for the extension of time. At the time of the Office Action, claims 1, 4-12, 14-17, 19-27, 30-33 and 37-51 were pending in the application.

### 1. Claim renumbering and amendments

Reconsideration of this Application and entry of the foregoing amendments are requested. Claims 1, 4-12, 14-17, 19-25, 27, 30-32, 36-44 and 47-51 have been cancelled and new claims 52-90 have been entered. Independent claims 52, 63, 64 and 75 recite a method to extract a total lipid fraction. Support for the amendments to these claims can be found, for example, at page 5, lines 5 to 7 and at page 8, lines 15 to 19. Further support for this terminology may be found throughout the disclosure where it is shown that the lipid fraction extracted from marine and aquatic animal is the total lipid fraction as may be seen more particularly in Table 13. Table 13 describes the various components of the lipids extracted according to the present claims which include triglycerides, free fatty acids, monoglycerides and phospholipids.

Claims 62, 63 and 64 do not recite "filleting-by-products". Support for this deletion may be found in the claims 15 and 16 as originally filed.

### II. Rejections Under 35 U.S.C. § 103

The Office Action rejected claims 1, 4-12, 14-17, 19-25, 27, 30-32, 36-44, 47-51 under 35 U.S.C. § 103(a) as being unpatentable over the disclosures of CA 2,155,571, WO 8401715, JP 360035057A and US 6,055,936 ("Collins") taken as a whole.

Prior to discussing the rejections, a brief presentation on the nature and constituents of lipids including those of marine and aquatic animal lipids is provided.

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### Lipids from animal and plant tissues

Within its generally recognized meaning and within the meaning of that which is recited by the claims, the term "lipid" refers to naturally occurring substances soluble in organic solvents, but insoluble in water. The diverse groups of substances encompassed by this definition can be divided into two broad classes: the acyl lipids and the terpenoids (minor components). The acyl lipids may then be subdivided into further subclasses: neutral acyl lipids (glycerides, free fatty acids and cholesterol esters), and polar lipids including alycerophospholipids, alyceroalycolipids and sphingolipids. Terpenoids are comprised of two subclasses of minor components: the sterols and the chlorophylls and carotenoids. Carotenoids constitute a very minor constituent of lipids in marine animals. The Examiner is referred to Table 17 on page 29 of the present application providing the content in astaxanthin and canthaxanthin of fractions obtained and to page 10, lines 26 to 31 where It is indicated that the content of astaxanthin is about 75-124µg/g and the cont nt of canthaxanthin is about 250-700 µg/g. It is apparent throughout the present application that the term "total lipid" as used therein is meant to encompass all these various types of lipids and that the method of the present invention is able to extract all these various types of lipids.

### Marine oils

The oils extracted from marine and aquatic materials can be separated into two types, according to their composition. The oils extracted from most fishes constitute the first oil type. It is composed of more than 90% of neutral acyl lipids, the major part of which are glycerides. Glycerides are fat reserves of marine animals. Cod liver oil for instance, is mostly constituted of glycerides.

The second oil type is composed of a lesser proportion of neutral acyl lipids and of glycerophospholipids, and sphingolipids. Krill lipids for instance may contain about 44% neutral acyl lipids (triglycerides, monoglycerides and free fatty acids) and about 54% of phospholipids (Phosphatidylcholines, Phosphatidylethanolamines, lysophosphatidylcholines

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and phosphatidylserines) and sphingolipids (sphingomyelin) (see table 13 of the present application).

Prior to the present invention, the only solvents that appeared to produce good results to extract total lipids from krill was a combination of chloroform and methanol. Notably, however, these solvents are unacceptable for the food industry, because after their evaporation, some toxic residues could remain in the lipids. There is no reported success of total lipids extraction with non-toxic solvents generally recognized as safe (GRAS). Furthermore, although acetone was used to extract certain components of lipids, it was never disclosed to extract total lipids from marine and aquatic animals.

## CA 2,155,571, JP 360035057A and Collin

It is respectfully submitted that JP 360035057A, Collin and CA 2,155,571, do not describe the use of acetone or ethanol for extracting "total lipids" within the meaning of the present application but to extract only a small fraction of total lipids, namely carotenoids for JP 360035057A and Collin, and polyunsaturated fatty acids for CA 2,155,571.

JP 360035057A and Collin teach that acetone is a conventional solvent for the recovery of fatty acids and lipid pigments (astaxanthin and canthaxanthin) of marine animals. Both polyunsaturated fatty acids and pigments constitute minor components of marine animal lipids. In the present application, the fraction 1 is a lipid fraction extracted with acetone according to a specific embodiment of the present invention. Analysis of lipid classes shows that free fatty acids constitute about 23.7% (See table 13) of total lipids of that fraction. The pigments, found in the hydrocarbon group (see Table 13) are only present as trace. As may further be seen from Table 17 of the present application, pigments constitute 0.0364% of the total lipid extracts of krill obtained according to the present invention (i.e. 93.1+270.4 =363.5µg/g oil). It is therefore submitted that techniques such as those described in JP 360035057A and Collin, which are able to extract less than 25% of total lipids, cannot be considered to be methods for total lipid extraction.

It is also respectfully submitted that it was not predictable from references such as those describing the extraction of minor components of marine animal lipids, namely

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polyunsaturated fatty acids and lipid pigments with acetone, that the remaining major components of lipids could also be extracted from marine animal with acetone. None of the cited references JP 360035057A, Collin or CA 2,115,571 disclose or suggest that acetone can efficiently extract glycerides, glycerophospholipids and sphingolipids from marine animals.

Notably, the cited prior art teaches away from using acetone to extract glycerophospholipids, sphingolipids and glycerides. Dawson et al., *Textbook of RMC*, Third Ed. "Data for Biochemical Research" and *The Merck Index*, 12th edition, Budavari et al., Ed. Merck Research Laboratories, 1996 teach that phosphatidylethanolamines, sphingomyelin and phosphatidylcholine are practically insoluble in acetone and that phosphatidylserine and lysophosphatidylcholine are only slightly soluble in acetone. The Table below presents the solubility of various glycerophospholipids and sphingolipids in acetone as disclosed in these references. Please find enclosed copies of the rel vant extracts of these references.

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Lipid	Reference	Solubility
Phosphatidylcholine	Dawson p.181	slightly soluble in acetone
(lecithin)		soluble in ethanol
	Merck #5452, p.925	Insoluble in acetone
		partially soluble in ethanol
Phosphatidylethanolamines	Dawson p.181	insoluble in acetone
(cephalin)		soluble in ethanol
	Merck #2022, p.328	insoluble in acetone
		slightly soluble in ethanol
Phosphatidylserines	Dawson p.183	insoluble in acetone
		insoluble in ethanol
Phosphatidylinositol	Dawson p.183	insoluble in acetone
		insoluble in ethanol
Sphingomyelin	Dawson p.184	insoluble in acetone
(sphingosine)		insoluble in ethanol
	Merck #8899, p.1495	insoluble in acetone
		insoluble in ethanol

It is also noteworthy that acetone is not mentioned as a potential solvent for triglycerides and that many of these neutral lipids are indicated to be only slightly soluble in ethanol. It is therefore respectfully submitted that at the time of the Applicants' invention, the prior art taught away from using acetone as a solvent for extracting lipids from marine animal. The Applicants themselves fortuitously discovered acetone's potential as a solvent for the extraction of krill lipids while they were investigating for a means to dehydrate krill tissues. In summary, the prior art does not disclose or suggest that the main classes of lipids (glycerophospholipids, sphingolipids and glycerides) can be efficiently extracted with acetone or ethanol.

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### Destruction of main components of krill lipids

JP 360035057A discloses an process by which krill is combined with an organic solvent such as acetone or hexane and a lipase. The main process seeks to decompose glycerides and phospholipids into fatty acids in order to achieve the concentration of carotenoids which can then be utilized as a dye. Now, as indicated earlier, marin and aquatic animals lipids contain glycerides, free fatty acids, phospholipids, glyceroglycolipids and sphingolipids. Each of these components are constituted in part of fatty acids. Thus, the method of JP 360035057A would damage the major components of marine and aquatic animals lipids.

For medical, cosmetic and nutritional applications, it is advantageous to preserve marine lipid integrity because they are then more easily assimilated in the organism. Hence, phospholipids for instance contribute to the formation of an emulsion with triglycerides during digestion, thereby facilitating lipase action which in turn may progressively release fatty acids and enable their good absorption by the organism. Furthermore, many if not all components of the total lipid extracts have nutritional values. A method able to extract the total lipids is therefore advantageous.

### WO 8401715

WO 8401715 describes a carbon tetrachloride (CCl<sub>4</sub>) extraction of enzymes from water-extracted lipids (see page 10, lines 4 to 10 of WO 8401715). CCl<sub>4</sub> is toxic according to the Merck Index (#1864, page 297 Merck Index, 12<sup>th</sup> edition): it may cause liver and kidney damage and may be a carcinogen.

In stark contrast, the method of the present invention is directed to an extraction of total lipids of marine and aquatic animals with a ketone solvent or a mixture of a ketone and an ethanol solvent or ethyl acetate. According to a specific embodiment of the present invention, active enzymes may be recovered from the solid residues produced. WO 8401715 does not disclose or suggest a method for extracting total lipids from marin and aquatic animals with a ketone solvent, the solvent used in WO 8401715 for extracting lipids is CCl<sub>4</sub>.

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In view of the above and foregoing, withdrawal of the rejection under 35 U.S.C. §103, first paragraph is respectfully requested. The rejections of the original claims are believed to have been overcome by the present remarks and the introduction of new claims. From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such action is earnestly solicited.

### III. Conclusion

Applicant has made every effort to present claims which distinguish over the prior art, and it is believed that all claims are in condition for allowance. Nevertheless, Applicant invites the Examiner to call the undersigned if it is believed that a telephonic interview would expedite the prosecution of the application to an allowance. In view of the foregoing remarks, Applicant respectfully requests reconsideration and prompt allowance of the pinding claims.

Respectfully submitted,

Date: 8-25-03

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Registration No. 25,931 Mark D. Passler

Registration No. 40,764

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Susan Bodavan, Editor Merck Research Laboratories Rahway, New Jersey 07065

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